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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/806,346	URTHALER ET AL.	
	Examiner	Art Unit	
	Laura McGillem	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *29 March 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-9,11-20,23 and 24* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9,12-15,17-20,23 and 24* is/are rejected.

7)☒ Claim(s) *11 and 16* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *23 March 2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *3/29/2007*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

It is noted that claims 1-3, 5, 7-8, 11-12, 14-16, 18, 20 and 23-24 have been amended and claims 10, 21-22, and 25-39 have been cancelled in the amendment filed 3/29/2007. Claims 1-9, 11-20 and 23-24 are under examination.

Specification

It is noted that the specification has been amended to correct trademark use. The objection to the specification has been withdrawn.

Claim Objections

It is noted that the claim 8 has been amended to correct spelling. The objection to the claim 8 has been withdrawn.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 13-14 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 13 is vague and indefinite because it recites the phrase "inlets that are situated close to each other" and the metes and bounds of "close" are not clear. The instant disclosure does not provide a limiting definition of "close to each other" other

than that the inlets should be "as close as possible" (see paragraph 0090). The skilled artisan would not know how close the inlets must be in order to meet the limitation of the claim. Claim 14 is indefinite insofar as it is dependent on an indefinite claim.

Claim 1 has been amended at the phrases "gently flow downward" and "reactor that is partially filled, in its lower part". Claim 7 has been amended to remove the phrase "the process". Claim 10 has been cancelled and claim 11 has been amended at the phrase "gently mixed". Claim 16 has been amended to remove the phrase "transportation between step b) and step d)". Claim 22 has been canceled. Claim 24 has been amended to remove the phrase "the cell mass".

The rejections of claims 1-9, 11-20 and 23-24 under 35 U.S.C. 112, second paragraph are withdrawn.

Claim Rejections - 35 USC § 102

It is noted that claim 1 has been amended at step b) to recite "disintegrating the cells by alkaline lysis by contacting the cell suspension with an alkaline lysis solution and allowing the cell suspension and the alkaline lysis solution to flow through a lysis reactor that is filled with particulate matérial, thereby forming a lysed cell solution. QIAGEN does not teach alkaline lysis solution flowing through a lysis reactor that is filled with particulate material. **Therefore the rejection of claims 1-2, 5, 7-9, 15, 17 and 19-20 under 35 U.S.C. 102(b) as being anticipated by The QIAGEN**

Transfection Source Book, 1999, pages 15 and 48-55 (herein, QIAGEN) is withdrawn.

It is noted that claim 1 has been amended at step b. Gonzales (U.S. Patent No. 5,783,686) does not teach a method comprising alkaline lysis solution flowing through a lysis reactor that is filled with particulate material. Therefore, the rejection of claims **1-5, 9, 15 and 19-20** under 35 U.S.C. 102(b) as being anticipated by Gonzales is withdrawn.

As noted above, claim 1 has been amended at step b). Marquet et al (WO 95/21250) does not teach a method comprising alkaline lysis solution flowing through a lysis reactor that is filled with particulate material. Therefore, the rejection of claims 1-2, 5-6, 9, 15 and 17-20 under 35 U.S.C. 102(b) as being anticipated by Marquet et al is withdrawn.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-2, 5, 7-9, 12, 15, 17 and 19-20 are rejected under 35 U.S.C. 103(a) as being unpatentable over The QIAGEN Transfection Source Book (herein,

QIAGEN, of record), in view of Santoro et al (U.S. Patent No. 6,660,472, filed 7/17/2000).

Briefly, Applicants claim a method for producing a biomolecule of interest in a host cell that is not secreted by the host cell, comprising the steps of: cultivating host cells, disintegrating the cells by alkaline lysis, precipitating the cell debris and impurities by neutralizing the lysate, separating the lysate from the precipitate and purifying the biomolecule of interest.

Applicants argued the use of the QIAGEN reference as anticipatory art under 35 U.S.C. 102(b) in the response filed 3/29/2007. Since the QIAGEN is being used in the rejection of claims under 35 U.S.C. 103(a), the argument will be addressed. Applicants submit that claim 1 has been amended to recite that the lysis reactor is directly connected to the neutralization reactor, and that the neutralization reactor is directly connected to the clarification reactor. Applicants submit that QIAGEN fails to teach such a structure and therefore fails to teach the method of Claim 1.

Applicant's arguments filed 3/29/2007 have been fully considered but they are not persuasive. The claimed method recites newly added limitations in step e) purifying the biomolecule of interest "wherein the lysis reactor is directly connected to the neutralization reactor and wherein the neutralization reaction is directly connected to the clarification reactor" which are structural limitations of an apparatus. These limitations do not appear to alter the method steps of the process of producing a biomolecule of interest comprising disintegrating cells by alkaline lysis to for a lysed cell solution, precipitating cell debris and impurities by neutralizing the lysed cell solution,

separating the lysate from the precipitate and purifying the biomolecule of interest. Therefore the teachings of QIAGEN are applicable to **claim 1**.

QIAGEN teaches a method to purify plasmid DNA from bacterial cultures. QIAGEN teaches the step of growing liquid cultures of bacteria comprising plasmids in selective LB medium for 12-16 hours at 37°C with shaking, followed by harvest by centrifugation and resuspension in a solution known as Buffer P1 (see steps 1-4 on page 49 and page 55, for example), which reads on cultivating host cells to produce a biomolecule of interest, harvesting and resuspending the cells to form a cell suspension.

QIAGEN teaches that a solution known as Buffer P2 comprising NaOH and SDS is added to the resuspended cell solution to produce a cell lysate (see page 49 step 5, and page 55), which reads on disintegrating the cells by alkaline lysis by contacting the cell suspension with an alkaline lysis solution. QIAGEN teaches that the P2 buffer should be added to the cell suspension and mixed gently and thoroughly by inversion and incubation at room temperature for five minutes. QIAGEN cautions to avoid shearing of genomic DNA (see page 49, step 5).

QIAGEN teaches that a solution known as Buffer P3, which contains potassium acetate at pH 5.5, is added to the lysate and causes the formation of a fluffy precipitate that comprises genomic DNA, protein, cell debris and SDS (see page 49 step 6 and page 55, in particular), which reads on precipitating the cell debris and impurities by neutralizing the lysed cell solution in a neutralization reaction to form a mixture comprising a precipitate and a lysate. Absent evidence to the contrary, any container in which the neutralization occurs would inherently be a neutralization reactor.

QIAGEN teaches that the mixture of lysate and precipitate is poured into an upright cartridge filter (i.e. clarification reactor) and air pressure is exerted from a plunger in order to flow the lysate downward through a filter (i.e. retention material).

QIAGEN illustrates a QIAfilter that appears to contain retention material in the lower part of the filter device (page 15), which meets the limitation of a clarification reactor partially filled with retention material. QIAGEN teaches that the lysate is recovered after the filtration (see page 49, steps 7-8, for example), which reads on separation of the lysate from a precipitate so that the precipitate and the lysate flow downward through a clarification reactor to retain the precipitate on top of and within the layer of retention material and wherein the cleared lysate leaves the reactor through the bottom.

QIAGEN does not teach that process comprising the step of disintegrating cells by alkaline lysis by contacting the cell suspension with an alkaline lysis solution and allowing the cell suspension and the alkaline lysis solution to flow through a lysis reactor that is filled with particulate material.

Santoro et al teach a method for lysing a bacterial microorganism to release a nucleic acid material, comprising a biological sample in a liquid medium placed in a container with at least one particulate material which is relatively hard and substantially inert compared to the nucleic material (see column 2, lines 40-48, for example). Santoro et al teach that the particulate material can consist of beads (see column 3, lines 24-25, for example). Santoro et al exemplify lysis of *S. epidermidis* cells in which a cellular suspension is added to beads, including iron or glass beads. Santoro et al teach that

the addition of the beads causes an increase in the percentage lysis and that the addition of iron or glass beads does not adversely affect the structure of the nucleic material released (see Example 4, column 12, lines 25-55, in particular).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method taught by QIAGEN to include a particulate material with the combination of cell suspension and alkaline lysis solution because Santoro et al teach that the addition of the beads causes an increase in the lysis percentage. The motivation to add beads is the expected benefit of being able to reduce or limit the time required for lysis of the biological sample (see Santoro et al column 4, lines 53-63, for example). There is a reasonable expectation of success to use beads in a cell disintegration step since it has worked previously in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention. Therefore the method of **claim 1** is rendered obvious the teaching of QIAGEN in view of Santoro et al.

QIAGEN further teaches that the filtered lysate is allowed to contact a positively charged anion-exchange resin by gravity flow so that plasmid DNA in the lysate binds to the resin (see page 50 step 11 and 13, for example), which reads on a lysate that contains the biomolecule of interest and meets the limitation of **claim 2**.

QIAGEN teaches that the mixture containing the precipitate and the lysate is filtered through a QIAfilter Maxi cartridge (see page 49, step 7-8, for example). QIAGEN

illustrates a QIAfilter that appears to contain retention material in the lower part of the filter device (page 15), which meets the limitation of a clarification reactor partially filled with retention material. Absent evidence to the contrary and a specific definition of the limitation of rigid, the QIAfilter is composed of a rigid retention material and meets the limitation of **claim 5**.

QIAGEN teaches that the mixture of lysate and precipitate is poured into an upright cartridge filter (i.e. clarification reactor) and air pressure is exerted from a plunger in order to flow the lysate downward through a filter (i.e. retention material). The insertion of the plunger into the clarification reactor would apply pressurized air in order to apply increasing pressure to the top of the lysate and precipitate mixture and thereby ensure a constant outflow of the lysate from the filter, and would meet the limitation of **claims 7-8**.

QIAGEN further teaches that the DNA on the resin is washed with Buffer QC to remove contaminants from the plasmid preparation (see page 560, step 11-12, for example) which reads on a washing step between the step of separating the lysate from the precipitate and purifying the plasmid of interest and meets the limitation of **claim 9**.

QIAGEN teach steps in which bacterial cells are harvested and pelleted into a centrifuge tube, and then resuspended in Buffer P1. Step 5 comprises the addition of Buffer P2 to lyse the cells (see page 49, steps 3-5, for example). Using the broadest reasonable interpretation of the recited limitation of introducing the cell suspension and the lysis solution into the lysis reactor in the form of two independent flows, QIAGEN procedure steps 3-5 meet the limitation of **claim 12**.

Instant claim 15 includes the limitation of a continuous mode step to mix the neutralizing solution with the lysed cell solution. QIAGEN teaches that the Buffer P3 (neutralizing solution) is added to the lysate solution by gentle inversions of the tube before adding the mix to the QIAfilter cartridge (clarification reactor). Absent evidence to the contrary, the lysed cell solution and the neutralizing solution would begin mixing when the neutralizing solution was added to the lysate, further mix during the inversions and would continue to mix during incubation in the Qiafilter cartridge until separation therefore meeting the limitation of mixing in a continuous mode and meet the limitation of **claim 15**.

QIAGEN teaches that a buffer known as QBT buffer that comprises NaCl, MOPS at pH 7.0 and isopropanol is added to the QIAGEN tip containing the anion exchange resin in order to equilibrate the resin (see page 50 step 10, for example). Absent evidence to the contrary, the lysate comprising the plasmid DNA would mix with the QBT buffer on addition to the column and alter some parameters of the lysate solution, such as salt composition, pH or conductivity and would therefore meet the limitation of a conditioning step as in **claim 17**.

QIAGEN teaches that plasmid DNA is eluted from the resin with a buffer known as Buffer QN and then precipitated from solution with isopropanol (see page 50, steps 11-14, for example), which reads on a step of purifying the biomolecule of interest such as a plasmid DNA as claimed in **claims 19-20**.

Claim 24 is rejected under 35 U.S.C. 103(a) as being unpatentable over The QIAGEN Transfection Source Book, (QIAGEN, of record) in view of Santoro et al (U.S. Patent No. 6,660,472) and further in view of Craig (U.S. Patent No. 6,381,967, of record).

In the response filed 3/29/2007, Applicants submit that Craig fails to suggest that the manual method for purifying nucleic acid in QIAGEN be altered such that the lysis reactor is directly connected to the neutralization reactor, so that the lysed cell solution is transported from the lysis reactor to the neutralization reactor via a direct connection. Applicants submit that Craig fails to suggest that the manual method of QIAGEN be modified such that the neutralization reactor is directly connected to the clarification reactor, so that the precipitate and lysate are transported from the neutralization reactor to the clarification reactor via a direct connection. Applicants submit that even in combination, QIAGEN and Craig would not suggest the method of claims 1 and 24.

Applicant's arguments filed 3/29/2007 have been fully considered but they are not persuasive. The claimed method recites newly added limitations in step e) that are structural limitations of an apparatus. These limitations do not appear to alter the method steps of the process of producing a biomolecule of interest comprising disintegrating cells by alkaline lysis to form a lysed cell solution, precipitating cell debris and impurities by neutralizing the lysed cell solution, separating the lysate from the precipitate and purifying the biomolecule of interest. Therefore the teachings of QIAGEN are applicable to independent claim 1 and dependent claim 24.

Applicants claim a method to purify a biomolecule of interest wherein a cell mass obtained by cultivating host cells to produce the biomolecule are cryo-pelleted.

The teachings of QIAGEN and Santoro et al are outlined in the above rejection. QIAGEN also teaches an optional method step in which the bacterial cells that have been harvested by centrifugation of the culture can be frozen in pellets for later use. QIAGEN or Santoro et al do not teach cryo-pelleting as disclosed in the specification as forming cryo-pellets by dropping the liquid material to be frozen into fluid gases and continuously bringing the resulting pellets out of the system.

Craig teaches problems that cause cell death during cell freezing, including death due to formation of large sharp ice crystals, and also cell poisoning due to osmotic dehydration by formation of ice crystals. Craig teaches that freezing can involve a process of vitrification, which is the solidification of solutions at low temperature without ice crystal formation. Craig teaches that the higher the speed of the temperature change, the lower the viscosity required to vitrify and faster freezing rates lead to smaller ice crystals (see column 1, lines 16-44, for example). Craig teaches that the goal of any cryopreservation process is to minimize cell damage (see column 2, lines 1-30, for example). Craig teaches a freezing method in which a liquid sample is transformed into small drops that are directly contacted with a partially solidified refrigerant. Craig teaches that this method is useful for substances that are susceptible to ice crystal or osmotic damage such as cells, plant material, tissue culture cells, sperm and embryos (see column 3, lines 20-25, column 4, lines 29-40, and column 12, lines 9-20, see, for example).

It would have been obvious to the skilled artisan at the time the invention was made to use the rapid freezing method as taught by Craig to form bacterial cell cryo-pellets for storage prior to a method of biomolecule purification as rendered obvious by QIAGEN and Santoro et al because QIAGEN suggests that the harvested bacterial cells can be frozen before use and Craig teaches an advantageous method of freezing cells. The motivation to freeze the bacterial cells by dropping them into partially solidified gas to form a cryopellet is the expected benefit of freezing the cells quickly in order to avoid formation of damaging and cytotoxic ice crystals as taught by Craig. There is a reasonable expectation of success to cryopellet bacterial cells before use since this has worked previously as taught by Craig. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 1-5, 9, 15, 19-20 and 23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gonzales (U.S. Patent No. 5,783,686, of record), in view of Santoro et al (U.S. Patent No. 6,660,472, filed 7/17/2000).

Applicants argued the use of the Gonzales reference as anticipatory art under 35 U.S.C. 102(b) in the response filed 3/29/2007. Applicants submit that at column 7, lines 26-50, Gonzales discuss the use of a Biomek automated workstation. Applicants submit that a Biomek workstation is a fluid handling robot that pipettes solutions. Applicants submit that it is different from the claimed method, in which the lysis reactor is directly

connected to the neutralization reactor, such that the lysed cell solution is transported from the lysis reactor to the neutralization reactor via a direct connection. Applicants submit that in the claimed method, the neutralization reactor is directly connected to the clarification reactor, such that the precipitate and lysate are transported from the neutralization reactor to the clarification reactor via a direct connection. Applicants submit that Gonzales fails to teach a method for producing a biomolecule of interest, in which the lysis reactor is directly connected to the neutralization reactor, or in which neutralization reactor is directly connected to the clarification reactor.

Applicant's arguments filed 3/29/2007 have been fully considered but they are not persuasive. The claimed method recites newly added limitations in step e) that do not appear to alter the method steps of the process of producing a biomolecule of interest comprising disintegrating cells by alkaline lysis to for a lysed cell solution, precipitating cell debris and impurities by neutralizing the lysed cell solution, separating the lysate from the precipitate and purifying the biomolecule of interest. Therefore the teachings of Gonzales are applicable to claim 1.

Gonzales teaches a method to isolate and purify nucleic acids such as plasmid DNA from bacterial cells or yeast lysates (see column 1, lines 16-30 and column 4, lines 22-35, for example). Gonzales teach that bacterial cell cultures are aerobically grown, pelleted by centrifugation and resuspended in a Tris Buffer solution (see column 7, lines 18-35, for example and page 55, for example), which reads on cultivating host cells to produce a biomolecule of interest, harvesting and resuspending the cells.

Gonzales teaches that a solution comprising NaOH and SDS is added to the resuspended cell solution to produce a cell lysate (see column 7, lines 36-39 and 44-46, for example), which reads on disintegrating the cells by alkaline lysis. Gonzales teaches that a solution that contains potassium acetate at pH 5, is added to the lysate to neutralize the mixture and causes the formation of a gummy mass that comprises chromosomal DNA and protein SDS complexes (see column 7, lines 48-52, in particular), which reads on precipitating the cell debris and impurities by neutralizing the lysate.

Gonzales teaches that the mixture of lysate and gummy mass is filtered in a well of a filter plate (e.g. clarification reactor) using a vacuum pressure to pull the cleared lysate through the filter to the well of a 96-well tissue culture plate, which retains the gummy mass (see column 7, lines 53-57, for example), which reads on separation of the lysate from a precipitate.

Gonzales teaches that DNA is recovered by elution with water (see column 8, lines 12-18, for example), which reads on a step of purifying the biomolecule of interest from the lysate.

Gonzales does not teach a process comprising the step of disintegrating cells by alkaline lysis by contacting the cell suspension with an alkaline lysis solution and allowing the cell suspension and the alkaline lysis solution to flow through a lysis reactor that is filled with particulate material, thereby forming a lysed cell solution.

Santoro et al teach a lysis method for a biological sample comprising a bacterial microorganism for releasing a nucleic acid material, comprising a biological sample in a

liquid medium placed in a container with at least one particulate material which is relatively hard and substantially inert compared to the nucleic material (see column 2, lines 40-48, for example). Santoro et al teach that the particulate material can consist of beads (see column 3, lines 24-25, for example). Santoro et al exemplify lysis of *S. epidermidis* cells in which a cellular suspension is added to beads including iron or glass beads. Santoro et al teach that the addition of the beads causes an increase in the lysis percentage and that the addition of iron or glass beads does not adversely affect the structure of the nucleic material released (see Example 4, column 12, lines 25-55, in particular).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method taught by Gonzales to include a particulate material with the combination of cell suspension and alkaline lysis solution because Santoro et al teach that the addition of the beads causes an increase in the percentage lysis. The motivation to add beads is the expected benefit of being able to reduce or limit the time required for lysis of the biological sample (see Santoro et al column 4, lines 53-63, for example). There is a reasonable expectation of success to use beads in a cell disintegration step since it has worked previously in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention. Therefore the method of claim 1 is rendered obvious by the teaching of Gonzales in view of Santoro et al.

Gonzales further teaches that silica slurry is added to the filtered lysate and allowed to incubate before another filtration through a filter plate, which retains the silica material (see column 7, lines 58-67, for example), and reads on a lysate that contains the biomolecule of interest and meets the limitation of claim 2.

Gonzales discloses a method in which glass beads are treated with hydrophilic material that would selectively bind proteinaceous material and not DNA. Gonzales discloses that DNA can be isolated by allowing DNA and protein containing mixtures to contact hydrophilic glass beads, and subsequently removing the beads to leave behind DNA in solution (see column 2, lines 9-16, for example), which reads on a clarification reactor with a retention material comprised of particulate material in the form of glass beads and meets the limitations of claims 3-4. Absent evidence to the contrary, the disclosed filter plate meets the limitation of a clarification reactor partially filled with retention material (Loprodyne/Loprosorb filter) because the upper part of the filter plate would necessarily have some volume to contain the mixture and the gummy precipitate. Absent evidence to the contrary and a specific definition of the limitation of rigid, the filter plate is composed of a rigid retention material and meets the limitation of claim 5. Gonzales teaches that the DNA on the silica is washed at least twice with an alcohol solution to remove unwanted material and impurities (see column 3, lines 1-3, column 6, lines 9-25 and column 8, lines 1-12, for example) which reads on a washing step between the step of separating the lysate from the precipitate to purify the plasmid of interest and meets the limitation of claim 9.

Gonzales teaches that the neutralizing solution is added to the lysate solution and reanneals the plasmid DNA. Absent evidence to the contrary, the lysed cell solution and the neutralizing solution would begin mixing when the neutralizing solution was added to the well, and would continue to mix during incubation in the well until separation, therefore meeting the limitation of mixing in a continuous mode as claimed in instant claim 15. Gonzales teaches that DNA is recovered from the silica by elution with water (see column 8, lines 12-18, for example), which reads on a step of purifying the biomolecule of interest such as a plasmid DNA from the lysate as in claims 19-20. Gonzales teaches that the method of plasmid DNA purification can be performed using a Biomek automated laboratory workstation (see column 7, lines 26-30 and 41-44, in particular), which reads on a method to purify plasmid DNA wherein at least one step is operated in an automated mode, absent evidence to the contrary the method would be in a continuous mode and meet the limitation of claim 23.

Claim 24 is rejected under 35 U.S.C. 103(a) as being unpatentable over Gonzales (U.S. Patent No. 5,783,686) in view of Santoro et al (U.S. Patent No. 6,660,472) and further in view of Craig (U.S. Patent No. 6,381,967, of record).

Applicants claim a method to purify a biomolecule of interest wherein a cell mass obtained by cultivating host cells to produce the biomolecule are cryo-pelleted.

Applicants submit that Craig fails to suggest that the method for purifying nucleic acid in Gonzales be altered such that the lysis reactor is directly connected to the neutralization reactor, so that the lysed cell solution is transported from the lysis reactor

to the neutralization reactor via a direct connection. Moreover, Applicants submit that Craig fails to suggest that the method of Gonzales be modified such that the neutralization reactor is directly connected to the clarification reactor, so that the precipitate and lysate are transported from the neutralization reactor to the clarification reactor via a direct connection. Applicants submit that even in combination Gonzales and Craig would not have suggested the method of Claims 1 and 24.

Applicant's arguments filed 3/29/2007 have been fully considered but they are not persuasive. The claimed method recites newly added limitations in step e). These limitations do not appear to alter the method steps of the process of producing a biomolecule of interest comprising disintegrating cells by alkaline lysis to for a lysed cell solution, precipitating cell debris and impurities by neutralizing the lysed cell solution, separating the lysate from the precipitate and purifying the biomolecule of interest. Therefore the teachings of Gonzales are applicable to independent claim 1 and dependent claim 24.

The teachings of Gonzales, and Santoro et al are outlined in the above rejection. Gonzales also teaches an optional method step in which the bacterial cells that have been harvested by centrifugation of the culture can be frozen in pellets for later use. Gonzales does not teach cryo-pelleting as disclosed in the specification as forming cryo-pellets by dropping the liquid material to be frozen into fluid gases and continuously bringing the resulting pellets out of the system. The teachings of Craig are discussed *supra*.

It would have been obvious to the skilled artisan at the time the invention was made to use the rapid freezing method as taught by Craig to form bacterial cell cryo-pellets to store cells prior to a method of biomolecule purification as taught by Gonzales in view of Santoro et al because Gonzales teaches that the harvested bacterial cells can be frozen before use and Craig teaches an advantageous method of freezing the cells. The motivation to freeze the bacterial cells by dropping them into partially solidified gas to form a cryopellet is the expected benefit of freezing the cells quickly in order to avoid formation of damaging and cytotoxic ice crystals as taught by Craig. There is a reasonable expectation of success to cryopellet bacterial cells before use since this has worked previously as taught by Craig. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 1-2, 5-6, 9, 15 and 17-20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Marquet et al (WO 95/21250, of record) in view of Santoro et al (U.S. Patent No. 6,660,472, filed 7/17/2000).

Applicants argued the use of the Marquet reference as anticipatory art under 35 U.S.C. 102(b) in the response filed 3/29/2007. Applicants submit that Marquet fails to teach a method for producing a biomolecule of interest, in which the lysis reactor is directly connected to the neutralization reactor, or in which neutralization reactor is directly connected to the clarification reactor.

Applicant's arguments filed 3/29/2007 have been fully considered but they are not persuasive. The claimed method recites newly added limitations in step e) that do not appear to alter the method steps of the process of producing a biomolecule of interest comprising disintegrating cells by alkaline lysis to for a lysed cell solution, precipitating cell debris and impurities by neutralizing the lysed cell solution, separating the lysate from the precipitate and purifying the biomolecule of interest. Therefore the teachings of *Gonzales* are applicable to claim 1.

Marquet et al teach a method for producing plasmid DNA. Marquet et al teach that plasmid DNA containing host bacterial cells are cultured, recovered and resuspended in buffer (see page 8, lines 11-21, and page 20, lines 10-35, for example), which reads on a step of cultivating host cells to produce a biomolecule of interest, harvesting and resuspending the cells. Marquet et al teach that the host cells are lysed by alkaline hydrolysis, and the lysate produced is subsequently acidified to facilitate removal of insoluble material (see page 8, lines 22-35 bridging to page 9, lines 1-6, for example), which reads on disintegrating the cells by alkaline lysis and precipitating the cell debris and impurities by neutralizing the lysate. Marquet et al teach that cell debris and impurities can be removed from the lysate containing DNA by filtration through a material that is porous enough for plasmid DNA to pass through, but not insoluble material. Marquet et al teach that the filter device can be comprised of a porous fritted glass disks. Marquet et al disclose that the filtration can be performed using gravity, pressure or vacuum (see page 9, lines 8-30, in particular), which reads on a step of

separating the precipitate from the lysate by allowing the mixture to flow downward using gravity through a clarification reactor that is partially filled with a rigid retention material where the precipitate is retained on top of and within the retention material and the lysate would flow through the bottom of the reactor. The skilled artisan would know that a gravity filtration device, such as a Buchner funnel (i.e. a clarification reactor) has the retention material or filter in the lower part of the funnel (see page 23, lines 14-20, for example).

Marquet et al does not teach a process comprising the step of disintegrating cells by alkaline lysis by contacting the cell suspension with an alkaline lysis solution and allowing the cell suspension and the alkaline lysis solution to flow through a lysis reactor that is filled with particulate material, thereby forming a lysed cell solution. The teaching of Santoro et al has been discussed above.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method taught by Marquet et al to include a particulate material with the combination of cell suspension and alkaline lysis solution because Santoro et al teach that the addition of the beads causes an increase in the percentage lysis. The motivation to add beads is the expected benefit of being able to reduce or limit the time required for lysis of the biological sample (see Santoro et al column 4, lines 53-63, for example). There is a reasonable expectation of success to use beads in a cell disintegration step since it has worked previously in the cited reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary

skilled artisan would have had a reasonable expectation of success in practicing the claimed invention. Therefore the methods of claims 1-2 are rendered obvious by the teaching of Marquet et al in view of Santoro et al.

Marquet et al teach that the filter can be non-flexible, porous fritted glass disks (see page 9, lines 26-28, for example), which meets the limitations of claims 5-6 of a rigid retention material or sinter plates as disclosed in the instant specification paragraph 0113. Marquet et al teach further step to precipitate and collect partially purified DNA using alcohol or PEG precipitation followed by filtration (see page 9, lines 30-35 and page 10, for example), which reads on a wash step before the purification step as in claim 9. Marquet et al teach that the neutralizing solution is added to the lysate solution. Absent evidence to the contrary, the lysed cell solution and the neutralizing solution would begin mixing when the neutralizing solution was added, and would continue to mix during incubation until separation by filtration, therefore meeting the limitation of mixing in a continuous mode in as claimed in instant claim 15. Marquet et al teach that washed precipitated plasmid DNA is resuspended in GRAS buffer or TE to alter pH and ionic strength (see page 9, lines 1-5, page 12, lines 27-30, page 20, lines 25-27 and page 21, lines 21-15, for example), which reads on a conditioning step and concentration step, wherein the concentration step takes place before the conditioning step as claimed in claims 17-18. Marquet et al teach that plasmid DNA is purified from impurities using size exclusion chromatography (see page 13, lines 10-35, for example), which meets the limitation of the claimed method wherein the biomolecule

of interest is a polynucleotide and plasmid DNA and meets the limitations of claims 19-20.

Claim 24 is rejected under 35 U.S.C. 103(a) as being unpatentable over Marquet et al (WO 95/21250) in view of Santoro et al (U.S. Patent No. 6,660,472) and further in view of Craig (U.S. Patent No. 6,381,967, of record).

Applicants claim a method to purify a biomolecule of interest wherein a cell mass obtained by cultivating host cells to produce the biomolecule are cryo-pelleted.

Applicants submit that Craig fails to suggest that the method for purifying nucleic acid in Marquet be altered such that the lysis reactor is directly connected to the neutralization reactor, so that the lysed cell solution is transported from the lysis reactor to the neutralization reactor via a direct connection. Moreover, Applicants submit that Craig fails to suggest that the method of Marquet be modified such that the neutralization reactor is directly connected to the clarification reactor, so that the precipitate and lysate are transported from the neutralization reactor to the clarification reactor via a direct connection. Applicants submit that even in combination, Marquet and Craig would not have suggested the method of Claims 1 and 24.

Applicant's arguments filed 3/29/2007 have been fully considered but they are not persuasive. The claimed method recites newly added limitations in step e). These limitations do not appear to alter the method steps of the process of producing a biomolecule of interest comprising disintegrating cells by alkaline lysis to for a lysed cell solution, precipitating cell debris and impurities by neutralizing the lysed cell solution, separating the lysate from the precipitate and purifying the biomolecule of interest.

Therefore the teachings of Marquet are applicable to independent claim 1 and dependent claim 24.

The teachings of Marquet and Santoro et al are outlined in the above rejection. Marquet et al also teaches an optional step in which the bacterial cells that have been harvested by centrifugation of the culture can be frozen and stored for later use. Marquet et al does not teach cryo-pelleting as disclosed in the specification as forming cryo-pellets by dropping the liquid material to be frozen into fluid gases and continuously bringing the resulting pellets out of the system. The teachings of Craig are discussed *supra*.

It would have been obvious to the skilled artisan at the time the invention was made to use the rapid freezing method as taught by Craig to form bacterial cell cryo-pellets to store cells prior to a method of biomolecule purification as taught by Marquet et al because Marquet et al teaches that the harvested bacterial cells can be frozen before use and Craig teaches an advantageous method of freezing the cells. The motivation to freeze the bacterial cells by dropping them into partially solidified gas to form a cryopellet is the expected benefit of freezing the cells quickly in order to avoid formation of damaging and cytotoxic ice crystals as taught by Craig. There is a reasonable expectation of success to cryopellet bacterial cells before use since this has worked previously as taught by Craig. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Conclusion

Claims 11 and 16 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Laura McGillem whose telephone number is (571) 272-8783. The examiner can normally be reached on M-F 8:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached on (571) 272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Laura McGillem, PhD
Examiner
6/20/2007

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